Biochemical Pharmacology, Vol. 23, pp. 1653–1657. Pergamon Press, 1974, Printed in Great Britain.

DISPLACEMENT BY URICOSURIC AGENTS OF SODIUM URATE BOUND TO HUMAN SERUM ALBUMIN

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(*Received* 8 *September* 1973; *accepted* 16 *November* 1973)

Abstract—The displacement of sodium urate by uricosuric agents from binding sites on human serum albumin (HSA) and normal serum has been demonstrated under physiological conditions *in vitro* (pH 7·4, ionic strength 0·16) over he oncentration range 1–13 mg/100 ml. At 37°, 0·5 mM sodium salicylate reduced urate bound to 5 g/100 ml of HSA from 19·6 to 9·7 per cent. At 22·5°, 22·6 per cent urate was bound to 5 g/100 ml of HSA, and this was reduced to 12·3 per cent by 0·5 mM salicylate, to 9·2 per cent by 0·5 mM phenylbutazone, to 14·6 per cent by 0·2 mM diflumidone, and to 17·9 per cent by 0·2 mM sulfaethidole. At 22·5°, pooled normal human serum (4·8 g/100 ml of albumin) bound 23·5 per cent urate; with 0·5 mM salicylate present 12·6 per cent urate was bound.

WE HAVE recently[1] demonstrated the binding of sodium urate to crystalline human serum albumin under physiological conditions. Previous reports[2–4] have suggested that part of the uricosuric action of some drugs is due to their interference with such binding, but drug displacement of urate has only been demonstrated indirectly or under conditions that are non-physiological. As the demonstration of displacement would be pertinent to the clearance of urate at the glomerulus and the mechanism of deposition of urate in tissues, the present study was undertaken to examine the displacement of sodium urate from albumin and serum by sodium salicylate, phenylbutazone, sulfaethidole and diflumidone, under physiological conditions *in vitro* of pH 7·4 and ionic strength 0·16.

EXPERIMENTAL

Urate binding and displacement was measured by the continuous ultrafiltration method of Blatt *et al.*[5] In initial experiments, the binding of urate to human serum albumin (HSA) and pooled normal serum was examined. The technique and calculations of binding in this method are as described by Campion *et al.*[1] Displacement of urate by competing drugs was then examined. This was accomplished in two steps. The albumin or serum was initially equilibrated with the competing drug. The drug (e.g. 0·5 mM sodium salicylate, 8 mg/100 ml, in phosphate buffer) was placed in the reservoir, and several times the chamber volume of this solution was passed through the ultrafiltration chamber which contained 0·724 mM (5 g/100 ml) HSA or pooled normal serum. In this way the reaction,

$$\text{protein} + \text{drug} \leftrightharpoons \text{protein} - \text{drug complex}$$

reached equilibrium. The reservoir was then refilled with a solution of sodium urate (0·79 mM, 15 mg/100 ml), containing sodium salicylate (or other competing drug) at the same concentration as just used to equilibrate the protein and connected to the chamber that now contained the salicylate-equilibrated HSA. During ultrafiltration as the free salicylate concentration and protein concentration remained constant, this salicylate–albumin equilibrium was maintained, and urate binding was studied under conditions of gradually increasing urate concentration. Results of binding with and without competing drug could then be directly compared. Full details of the steps for ultrafiltration, analysis and methods for establishing displacement are described in the paper by Campion and Olsen.[6] Because of its greater convenience, many experiments were done at room temperature (22·5°).

Sodium urate was prepared from uric acid titurated with 0·1 N NaOH and then made up with phosphate buffer to a concentration of 0·79 mM (15 mg/100 ml). The ionic strength of the final solution was 0·16, and pH 7·4. Urate determination was performed using the method of Liddle *et al.*[7]

Human crystalline albumin (CalBiochem, crystalline human serum albumin, electrophoretically pure, defatted) in a solution of 5 g/100 ml (0·724 mM) was also prepared in phosphate buffer, ionic strength 0·16, pH 7·4. Normal serum was pooled from several healthy male volunteers. The albumin concentration of the pooled serum, measured by microzone protein electrophoresis, was 4·8 g/100 ml (0·695 mM). In order to remove naturally present uric acid from the serum, it was washed with phosphate buffer by ultrafiltration until uric acid in the pooled serum was zero. An additional experiment, using 5 g/100 ml (0·724 mM) Amour laboratories DBS-MSA albumin, was performed to test whether the presence of the large amount of the fatty acid caprylate used as preservative reduced the binding capacity of the albumin.

RESULTS

With buffer alone in the ultrafiltration chamber, the concentration of urate in the ultrafiltrate was always equal to calculated total urate, indicating that urate was

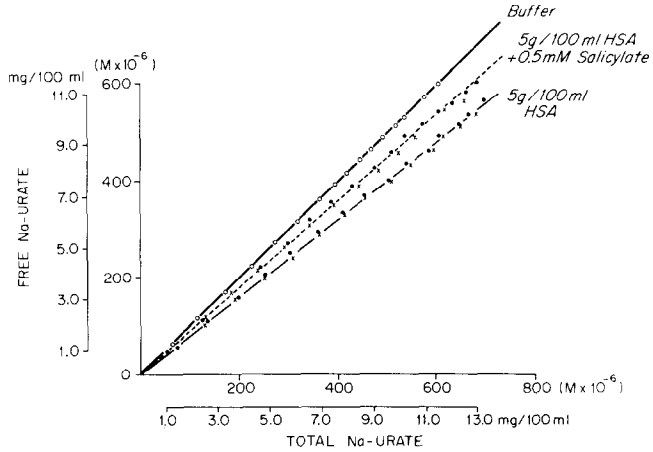

FIG. 1. Binding of Na-urate by 0·724 mM (5 g/100 ml) human serum albumin at pH 7·4, ionic strength 0·16, 38°. Symbols indicate: O——O, phosphate buffer; ×●——●×, two separate experiments with 0·724 mM albumin; and ×●- - - -●×, two separate experiments with 0·724 mM albumin and 0·5 mM Na-salicylate.

freely filtered by the membrane. With 0·724 mM (5 g/100 ml) HSA in the chamber, a constant fraction of total urate was bound ($r = 0·99$). The percentage of urate bound over the concentration range 0·053 to 0·684 mM (1–13 mg/100 ml) was 19·6 per cent (S.E. ± 0·3, n = 32) at 37° and 22·6 per cent (S.E. ± 0·3, n = 78) at 22·5°. In the presence of 0·5 mM (8 mg/100 ml) sodium salicylate, there was a 51 per cent reduction in binding at 37° and a 46 per cent reduction at 22·5°. The effect of added salicylate on binding is demonstrated in Fig. 1, the reduction being significant [regression analysis ($P < 0·01$)].

TABLE 1. EFFECT OF SODIUM SALICYLATE, PHENYLBUTAZONE, DIFLUMIDONE AND SULFAETHIDOLE ON THE BINDING OF URATE TO 0·724 mM (5 g/100 ml) CRYSTALLINE HUMAN SERUM ALBUMIN (pH 7·4, IONIC STRENGTH 0·16)

	22·5°		37°	
	Na-urate bound (%)	Reduction in binding (%)	Na-urate bound (%)	Reduction in binding (%)
Control	22·6 ± 0·3 (78)*		19·6 ± 0·3 (32)	
Sodium salicylate (0·5 mM)	12·3 ± 0·2 (32)	46	9·7 ± 0·3 (32)	51
Phenylbutazone (0·5 mM)	9·2 ± 0·4 (16)	59	ND†	ND
Diflumidone (0·2 mM)	14·6 ± 0·2 (16)	36	ND	ND
Sulfaethidole (0·2 mM)	17·9 ± 0·2 (16)	21	ND	ND

* Mean ± S.E.M. (number of observations).
† ND = not done.

The pooled normal sera (albumin concentration of 0·695 mM, 4·8 g/100 ml) bound 23·5 per cent (S.E. ± 0·7, n = 12) of the urate at 22·5°; this was reduced significantly by 0·5 mM salicylate to 12·6 per cent (S.E. ± 0·7, n = 12), regression analysis ($P < 0·01$).

Phenylbutazone (0·5 mM, 15·3 mg/100 ml), diflumidone (0·2 mM, 6·7 mg/100 ml) and sulfaethidole (0·2 mM, 5·7 mg/100 ml) also significantly displaced urate (Table 1) from HSA.

A 5 g/100 ml (0·724 mM) Armour albumin solution bound a constant ($r > 0·99$) 11·3 per cent (S.E. ± 0·5, n = 13) of urate over the concentration range of 0·053 to 0·684 mM (1–13 mg/100 ml) sodium urate.

DISCUSSION

A previous study demonstrated the binding of a constant fraction of sodium urate to crystalline human serum albumin under physiological conditions *in vitro*. Over the range of 0·053 to 0·684 mM (1–13 mg/100 ml), 22·6 per cent of sodium urate was bound at 22·5°, and 19·6 per cent was bound at 37°, to 5 g/100 ml of HSA. The present study is extended to pooled normal sera which had an albumin concentration of 0·695 mM (4·8 g/100 ml), and bound 23·5 per cent of sodium urate at 22·5°. HSA used in these and previous studies has had removed virtually all of the fatty acids that strongly bind to albumin. The albumin of the washed pooled sera still had normal amounts of bound fatty acid,[8] and at an albumin concentration equal to that in the HSA studies, the serum albumin bound 23·5 per cent of urate, which was almost identical to the 22·6 per cent bound by the defatted HSA. We can, therefore,

conclude that at normal fatty acid concentrations, there is little sharing of the albumin's fatty acid and urate binding sites. A clear decrease in urate binding to HSA occurred in the presence of 0·5 mM (8 mg/100 ml) sodium salicylate (46 per cent reduction in binding) (Fig. 1), 0·5 mM (15·3 mg/100 ml) phenylbutazone (59 per cent), 0·2 mM (6·7 mg/100 ml) diflumidone (36 per cent) and 0·2 mM (5·7 mg/100 ml) sulfaethidole (21 per cent). Similarly, 0·5 mM salicylate caused a 53 per cent reduction in binding of urate by albumin of pooled normal serum. Therefore, these drugs did effectively displace urate from both HSA which was defatted and from pooled normal serum, indicating effective sharing of the binding sites of these compounds with urate. Comparison of the association constants (k) of urate with that of salicylate and phenylbutazone as determined on defatted crystalline HSA[1,9] indicates that the k values of these displacing compounds are at least two orders of magnitude higher than urate. At 22·5°, urate has an association constant of $4{\cdot}47 \times 10^2 M^{-1}$ (assuming one binding site), whereas salicylate has a k_1 equal to $1 \times 10^5 M^{-1}$, and phenylbutazone has a k_1 of $1 \times 10^5 M^{-1}$. The association constant for sulfaethidole and bovine serum albumin is $1{\cdot}2 \times 10^5 M^{-1}$.[10] Appropriate association constant for diflumidone is not available.

Albumin solutions prepared commercially for intravenous albumin replacement therapy commonly have large concentrations of fatty acids such as caprylate added as preservative. Fatty acids have high association constants and have been shown[11] to displace many compounds. It is, therefore, not surprising that the commercially available albumin solutions bound only half of the usual amount of urate. When using such solutions as replacement therapy, it is important to remember that while the oncotic action of albumin may be unimpaired, its secondary transport function may be considerably diminished.

If this demonstration of displacement from binding sites *in vitro* also occurs *in vivo*, then a decrease in binding of approximately 50 per cent would increase the clearance of urate at the glomerulus. This has been in part the explanation for our previous demonstration of uricosuric action of these drugs,[2,4] and it is now substantiated by the present data with displacement under physiological conditions *in vitro*. If the albumin–drug interaction is a model of drug–receptor interaction,[12] then the binding of urate by albumin and its displacement by drugs as described in this study would suggest that the deposition of urate in tissue could be modified by appropriate drug administration.

Acknowledgements—The expert technical assistance of Messrs. Robert Olsen, Jerry Jacobson and Willie Mills is gratefully acknowledged. Supported by USPHS grant GM 15759, VA Research Funds and a grant from the Kroc Foundation.

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